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Effective Immunization against *Bordetella pertussis* Respiratory Infection in Mice Is Dependent on Induction of Cell-Mediated Immunity

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A murine respiratory challenge model was used to examine the induction of cellular and humoral immune responses and their role in protection against *Bordetella pertussis* following immunization or previous infection. Spleen cells from mice convalescing from a *B. pertussis* infection exhibited extensive in vitro T-cell proliferation and secreted high levels of interleukin-2 (IL-2) and gamma interferon but not IL-4 or IL-5, a cytokine profile typical of $CD4^+$ Th1 cells. Serum from these mice had low or undetectable anti-*B. pertussis* antibody levels. In contrast, mice immunized with an acellular pertussis vaccine had high levels of *B. pertussis* antibodies and spleen cells secreting IL-5 but not gamma interferon, a profile characteristic of $CD4^+$ Th2 cells. Immunization with an inactivated whole-cell vaccine induced both $CD4^+$ Th1 and serum antibody responses. After exposure to a *B. pertussis* respiratory challenge, the convalescent mice and those immunized with the whole-cell vaccine eliminated the bacterial infection significantly faster than mice immunized with the acellular vaccine. These findings show that the selection of antigens and their form of presentation are important in determining whether the subsequent immune response is cellular, mediated by Th1 cells, or humoral, mediated by Th2 cells. In the murine model, the induction of a Th1-mediated cellular immune response appears to be a key element in acquired immunity to a *B. pertussis* infection.

Whooping cough (pertussis), a respiratory disease caused by *Bordetella pertussis*, accounts for more than 300,000 deaths annually worldwide (15). A killed whole-cell vaccine (WCV) has been available in many countries for over 40 years, and while its use seems to control pertussis epidemics, the nature of the immunity that it induces is still uncertain, and there are some doubts concerning its safety. Respiratory infection of mice, achieved by intranasal instillation (10) or aerosol challenge (43) with *B. pertussis*, is now a widely used model for study of the pathogenesis and immunology of this disease (29, 30, 36, 38). This model, when it incorporates adult immunocompetent mice, is a nonlethal model with many similarities to infection observed in infants (44). In the murine model, antibodies against the two major putative protective antigens, pertussis toxin (PT) and filamentous hemagglutinin (FHA), acquired by active immunization or passive transfer, can protect against infection (17, 20, 42). However, a recent Swedish clinical trial of acellular pertussis vaccines failed to demonstrate a correlation between protection against clinical disease and levels of serum antibodies to PT or FHA (1).

It is now known that *B. pertussis* is capable of invading eukaryotic cells (11), and it has been found within alveolar macrophages from AIDS patients (4). It has been suggested that cell invasion by and intracellular survival of *B. pertussis* may be mechanisms for persistence (13, 45). Recent reports have demonstrated the presence of T cells specific for *B. pertussis* components in humans and mice following infection (9, 16, 23). Using the murine respiratory infection model, we previously demonstrated that the adoptive transfer of $CD4^+$ T cells from immune mice can confer protection against an aerosol challenge in the absence of a detectable serum antibody response (24). Furthermore, we and others demonstrated that the T cells induced following infection secrete interleukin-2 (IL-2) and gamma interferon (IFN-γ) but not IL-4 or IL-5, a cytokine profile characteristic of Th1 cells (24, 34). Taken together, these findings demonstrate a role for cell-mediated immunity (CMI) in pertussis respiratory infection in mice and strongly suggest its involvement in recovery from and prevention of disease in humans.

In the present study, we examined antibody, T-cell proliferative, and cytokine responses to a range of *B. pertussis* antigens by using mice that had been immunized with either a killed WCV or an acellular vaccine comprising chemically detoxified PT (PTd), FHA, and the 69-kDa protein pertactin or mice that had recovered from a *B. pertussis* respiratory infection. The protection afforded by the different treatments was assessed by aerosol challenge of the mice with virulent *B. pertussis* and examination of the progression of the infection by use of viable counts of bacteria in the lungs. Our findings demonstrated that the choice of immunogen can determine the induction of cellular immunity, mediated by Th1 cells, or humoral immunity, stimulated by Th2 cells, which in turn can determine the level of protection against respiratory challenge. High antibody levels appeared to result in an early decline in the number of viable organisms in the lungs, but complete clearance was dependent on CMI.

MATERIALS AND METHODS

Mice. Female BALB/c mice were bred and maintained at National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom, under specific-pathogen-free conditions. All mice were 8 to 12 weeks old at the initiation of experiments.

Bacterial antigens. A formaldehyde-treated sonicate of

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B. pertussis Wellcome 28 (W28) was prepared as previously described (24). In brief, a bacterial suspension (5×10^7 to 5×10^9 cells per ml) in phosphate-buffered saline (PBS) was sonicated, the debris was removed by centrifugation, and the protein concentration was adjusted to 50 to 100 μg/ml. Gelatin (0.02%) and Tween 20 (0.05%) were added, and the material was treated with 0.2 to 0.4% formaldehyde for 7 days and then dialyzed exhaustively against PBS. Killed *B. pertussis* W28 cells were prepared by heating of a bacterial suspension in PBS at 80°C for 30 min. Purified native PT, FHA, and pertactin and PTd, prepared by treatment with glutaraldehyde and then formalin, from *B. pertussis* Tohama were kindly provided by Carine Capiau, SmithKline Beecham, Rixensart, Belgium (5, 41). An acellular pertussis vaccine was prepared to contain 25 μg each of FHA, pertactin, and PTd (supplied by SmithKline Beecham) adsorbed to 1.0 mg of aluminium sulfate per ml of PBS. The third British reference preparation for pertussis vaccine (88/522) was used as the WCV (37).

Aerosol infection. Respiratory infection of mice was initiated by aerosol challenge by a modification of the method described by Sato et al. (43). *B. pertussis* W28 phase I was grown under agitation conditions at 36°C in Stainer-Scholte liquid medium (49). Bacteria from a 48-h culture were resuspended at a concentration of approximately 2×10^{10} CFU/ml in physiological saline containing 1% casein. Mice were exposed for 12 min to the challenge inoculum, administered as an aerosol by use of a nebulizer in a container within a class 3 exhaust-protected cabinet. Four mice were killed 2 h after aerosol challenge to assess the initial numbers of viable *B. pertussis* in the lungs.

Enumeration of viable bacteria in the lungs. Lungs were removed aseptically and homogenized in 1 ml of sterile physiological saline with 1% casein on ice. Drops (20 μl) of a serially diluted homogenate from individual lungs were spotted in triplicate onto each of three Bordet-Gengou agar plates. When the numbers of viable bacteria per lung were low, 100-μl portions of an undiluted homogenate were spread onto each of three Bordet-Gengou agar plates. Results are reported as the mean viable *B. pertussis* counts for individual lungs from four mice. The limit of detection was approximately $\log_{10}$ 0.5 CFU per lung.

Immunizations. Immunized mice each received two intraperitoneal injections comprising either half a human dose (2 IU) of the plain WCV (88/522) in 0.5 ml of PBS or 0.5 ml of the acellular vaccine (12.5 μg each of FHA, pertactin, and PTd with 0.5 mg of aluminium sulfate) 4 weeks apart and were examined or challenged 2 weeks after the second immunization. Mice that were infected by a standard aerosol challenge with *B. pertussis* W28 and allowed to recover for 6 weeks, by which time they were completely free of the bacteria, formed the convalescent group. Control mice received no treatment.

Serum and lung antibody determinations. The levels of antibodies to *B. pertussis* components in sera and lung homogenates were estimated by use of an enzyme-linked immunosorbent assay (22). Pertussis sonicate (5 μg/ml) and PT, FHA, and pertactin (2 μg/ml each) were used to coat microtiter plates. A serum pool derived from mice 42 days after immunization with the WCV (88/522) (2 IU on days 0 and 28) was used as a reference. Serum and lung samples were centrifuged and diluted in PBS–0.05% Tween 20 prior to the assay. Bound antibodies were detected by use of alkaline phosphatase-conjugated anti-mouse immunoglobulin G (IgG), IgM, IgA, and IgE (Sigma Chemical Co., Poole, Dorset, United Kingdom). Levels of serum antibodies to each antigen preparation were estimated by assigning a nominal value of 100 U/ml to the reference serum pool. Antibody levels, expressed in units per milliliter of undiluted serum, were determined from the reference by use of a parallel-line assay. Lung homogenate IgA antibody levels were expressed as endpoint titers following dilution of original samples prepared in 1 ml of PBS.

Proliferation assay. Spleens were removed, and a single-cell suspension was prepared in RPMI 1640 medium supplemented with 2% normal mouse serum. Cells (2×10^6/ml) were incubated with heat-killed *B. pertussis* cells (10^6/ml), formaldehyde-treated sonicate (5 μg/ml), PT (0.2 μg/ml), FHA (1 μg/ml), and pertactin (5 μg/ml) as previously described (23). The proliferative responses, determined after 4 days on the basis of [^{3}H]thymidine incorporation, were measured by scintillation counting, and the results were expressed as mean counts per minute for triplicate cultures.

Lymphokine assays. Spleen cells were cultured in the presence of *B. pertussis* antigens, and the supernatants were removed after the optimum times for lymphokine secretion, 24 h for IL-2 or 48 h for IL-5 and IFN-γ, and stored at −20°C until assayed. IL-2 levels were assessed by measuring the growth of an IL-2- or IL-4-dependent cell line, CTLL-2, in the presence of anti-IL-4 antibody IIBII, and the results were expressed as mean counts per minute for triplicate cultures. IFN-γ and IL-4 levels were assayed by use of an immunoradiometric assay and an immunoassay, respectively, as previously described (52); IFN-γ levels were expressed as units per milliliter relative to a reference preparation. IL-5 levels were assessed by use of an immunoassay with anti-mouse IL-5 monoclonal antibody TRFK5 for capture and biotinylated rat anti-mouse IL-5 monoclonal antibody TRFK4 (PharMingen, San Diego, Calif.) for detection, as described by Schumacher et al. (46). Murine recombinant IL-5 (PharMingen) was used for the generation of the standard curve.

Statistical methods. Antibody, proliferation, and cytokine secretion data from the different treatment groups were compared by use of *t* tests.

RESULTS

Protective efficacies of immunization and previous infection. Six weeks after the initiation of primary infection or immunization, mice were infected in the respiratory tract by an aerosol challenge with *B. pertussis*. This resulted in a reproducible initial colonization level of approximately 3×10^5 CFU per lung (Fig. 1). During the first 5 days of infection, nonimmunized control mice showed an increase in the number of viable bacteria of up to 100-fold. After this time, the number of bacteria in the lungs slowly declined, although more than 10^3 CFU per lung was still detectable 15 days after infection, but bacteria were undetectable 35 days after the challenge (Fig. 1 and data not shown). In contrast, in all three treatment groups, the numbers of bacteria declined soon after the challenge. Two days after the challenge, both WCV- and acellular vaccine-immunized mice showed reductions in the bacterial load that were more than 10-fold greater than that in the convalescent mice ($P < 0.001$). However, 5 days after the challenge, no bacteria were detectable in the lungs of convalescent mice or mice immunized with the WCV, whereas mice immunized with the acellular vaccine required much longer to clear the infection and still harbored viable bacteria 8 days after infection.

Antibody levels at the time of challenge. Six weeks after the

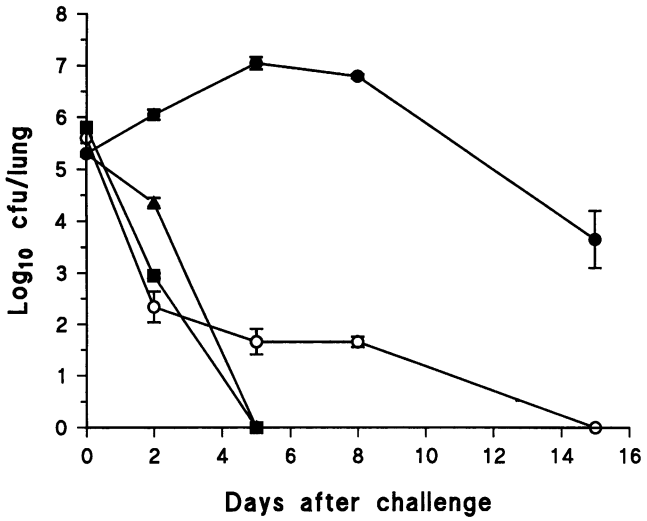

FIG. 1. Course of *B. pertussis* respiratory infection in convalescent and immunized mice. Infections initiated at day 0 were monitored by performing viable counting of bacteria in the lungs at intervals after the challenge. Groups were convalescent (■), WCV-immunized (▲), acellular vaccine-immunized (○), and unimmunized control (●) animals. The results are reported as mean CFU per lung estimated for individual lungs from four mice for each group at each time point. Bars represent standard errors of the means.

initiation of infection or immunization, which coincided with the time of the aerosol challenge, the antibody responses of the mice were assessed against a range of *B. pertussis* antigens (Fig. 2a). Levels of anti-*B. pertussis* IgG antibodies in the convalescent mice were low, indicating only a weak response to PT and no detectable responses to the other antigens. Mice immunized with the WCV had levels of IgG to the *B. pertussis* sonicate and to all of the individual antigens that were significantly higher than those of the convalescent mice ($P < 0.001$). Mice immunized with the acellular vaccine had very high levels of serum antibodies to all three individual antigens, higher than those of convalescent mice ($P < 0.001$) or WCV-immunized mice ($P < 0.05$), but no measurable response to the sonicate. The serum IgM responses (data not shown) in all the treatment groups were generally low and reflected the distribution and relative magnitudes of the IgG results; i.e., the highest levels were seen in the acellular vaccine-immunized mice and the lowest levels were seen in the convalescent mice. Serum IgA and IgE responses were below the level of detection, and no antibody responses to any of the test antigens were observed in nonimmunized control mice.

Secretory IgA levels in lung homogenates (Fig. 2b) also revealed that the strongest response, in particular to FHA, was generated with the acellular vaccine ($P < 0.001$ compared with convalescent mice and WCV-immunized mice). Mice immunized with the WCV had a low level of mucosal anti-FHA IgA in the lungs, and convalescent mice showed weak secretory IgA responses to PT and FHA.

Antibody responses following a *B. pertussis* challenge. The serum and lung antibody levels were assessed 5 days postchallenge, when bacteria were no longer detectable in the lungs of the convalescent and WCV-immunized mice, and 15 days postchallenge, when the acellular vaccine-immunized mice had also cleared the infection (Fig. 3). There was no significant change in the levels of serum IgG antibodies against the *B. pertussis* sonicate, FHA, and pertactin in any of the mice following the challenge ($P > 0.05$). There were small but significant ($P < 0.001$) increases in both the serum IgG and the lung IgA antibody levels to PT in the convalescent mice. These mice also showed an anamnestic IgA response to the *B. pertussis* sonicate that appeared at day 5 and reached a titer of 200 by day 15 ($P < 0.001$). However, the IgA levels against FHA were reduced in all treatment groups following the challenge. The nonimmunized control mice still had no detectable antibody responses to any *B. pertussis* antigens 15 days after the primary infection.

Proliferative responses of spleen cells to *B. pertussis* antigens. The in vitro proliferative responses of antigen-stimulated spleen cells taken from mice at the time of the challenge revealed marked differences among the groups (Fig. 2c). Cells from convalescent mice showed the greatest proliferative responses, particularly when stimulated with killed *B. pertussis* or detoxified sonicate ($P < 0.001$, in comparison with cells from WCV- or acellular vaccine-immunized mice). Spleen cells from mice immunized with the WCV showed moderate responses to *B. pertussis* whole bacteria and the sonicate, and those from mice immunized with the acellular vaccine showed poor responses to the sonicate and moderate responses to the whole bacteria, FHA, and PT. Although pertactin stimulated spleen cells from convalescent mice, it failed to induce significant proliferation of spleen cells from immunized mice. Spleen cells from nonimmunized controls showed no significant proliferation in response to any of the antigens.

Lymphokine secretion of spleen cells. The in vitro lymphokine secretion profiles of antigen-stimulated spleen cells revealed further contrasts among the groups (Fig. 4). Cells derived from convalescent mice secreted large to moderate amounts of IL-2 in response to in vitro stimulation with killed *B. pertussis*, the sonicate, PT, FHA, or pertactin. These cells also released moderate amounts of IFN-γ when stimulated with killed *B. pertussis* but only small amounts in response to stimulation with the other antigens, and their IL-5 responses to all the antigens were low.

Spleen cells from mice immunized with the WCV secreted reasonable levels of IL-2 in response to stimulation with all *B. pertussis* antigens except for pertactin. These cells released large amounts of IFN-γ following stimulation with killed *B. pertussis* whole bacteria, moderate amounts following stimulation with the *B. pertussis* sonicate, but very little following stimulation with the other antigens. However, only low levels of IL-4 or IL-5, not significantly higher than those produced by nonimmunized control mouse spleen cells ($P > 0.1$), were produced in response to any of the antigenic stimuli. In contrast, cells derived from the spleens of acellular vaccine-immunized mice secreted little or virtually no IL-2 or IFN-γ upon in vitro stimulation with any of the *B. pertussis* antigens. However, these cells did produce low levels of IL-4 (50 to 100 pg/ml; data not shown) and levels of IL-5 that were significantly higher than those produced by cells from control, convalescent, or WCV-immunized mice in response to all antigens except for the *B. pertussis* sonicate ($P < 0.01$ to $P < 0.001$). Furthermore, T-cell lines derived from mice immunized with the acellular vaccine produced high levels of IL-4 and IL-5 (2).

DISCUSSION

The results of this study show a relationship between the development of *B. pertussis*-specific CMI and acquired resistance to a *B. pertussis* respiratory infection. The T-cell-

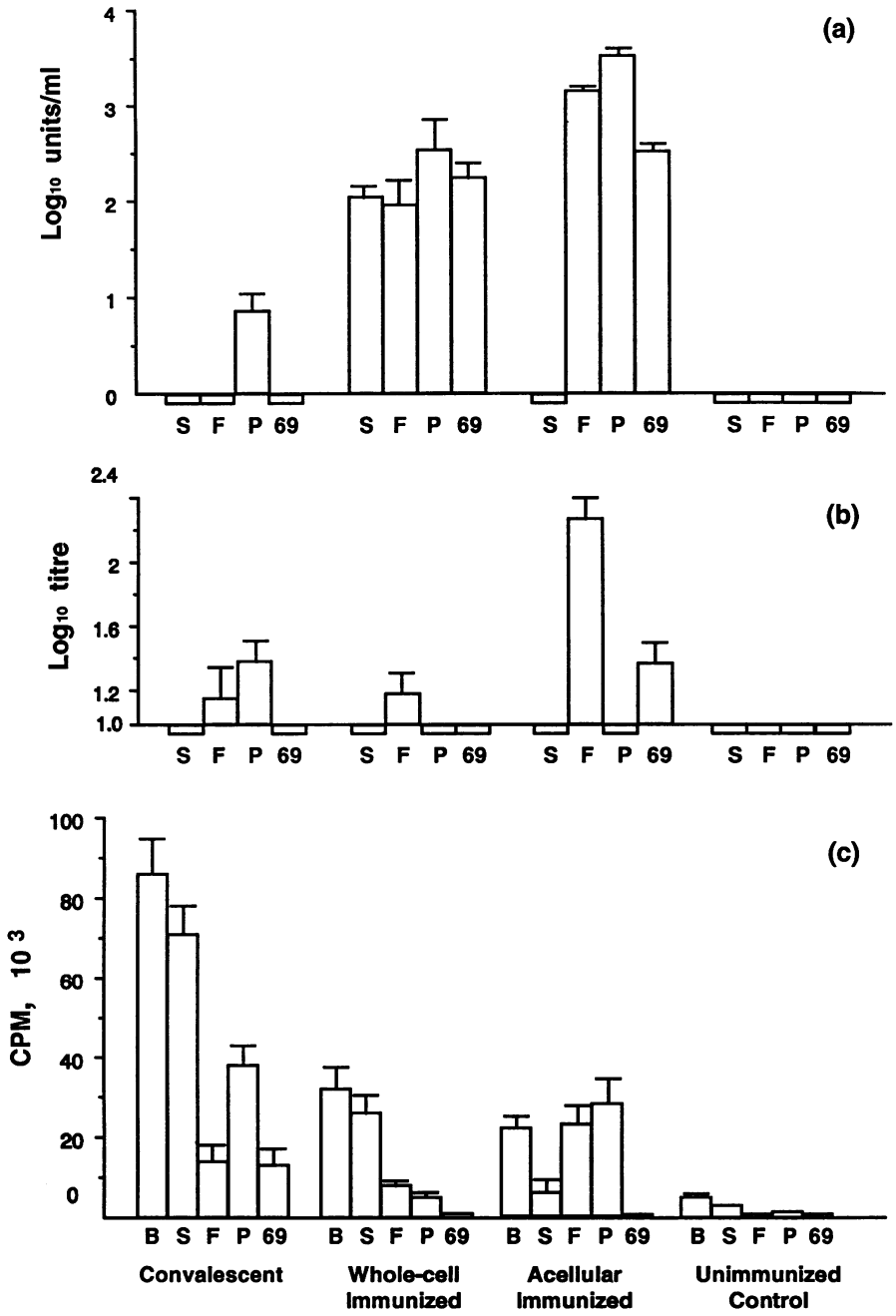

FIG. 2. Antibody and T-cell responses to *B. pertussis* components at the time of the aerosol challenge. Shown are serum IgG (a), lung IgA (b), and in vitro spleen cell proliferative (c) responses of convalescent, WCV-immunized, acellular vaccine-immunized, and nonimmunized mice to *B. pertussis* sonicate (S), FHA (F), PT (P), pertactin (69), or killed *B. pertussis* cells (B). A minimum of six mice from each group were assayed. Bars represent standard errors of the means.

mediated response may be induced as a result of infection or immunization with the WCV and, when induced by infection, is accompanied by only minimal antibody responses. This result is in agreement with our previous report indicating the critical role of cellular immunity in the clearance of a *B. pertussis* primary infection from the murine respiratory tract (24) and also demonstrates the importance of T cells in protective immunity acquired by immunization.

Until recently, pertussis was considered to be a noninvasive mucosal infection giving rise to a toxin-mediated disease (35). Consistent with this view, most investigations of immunity to the disease have centered on humoral responses, particularly the levels of serum IgG, to selected antigens, mainly PT and putative adhesins (6, 29, 39, 42). However, more recent findings demonstrating the invasive potential of *B. pertussis* (11) and the presence of antigen-specific T-cell responses following infection (9, 24, 34) have necessitated a reevaluation of potential protective mechanisms.

As previously reported by us and others (24, 34), the time courses for the development of cellular and humoral immune

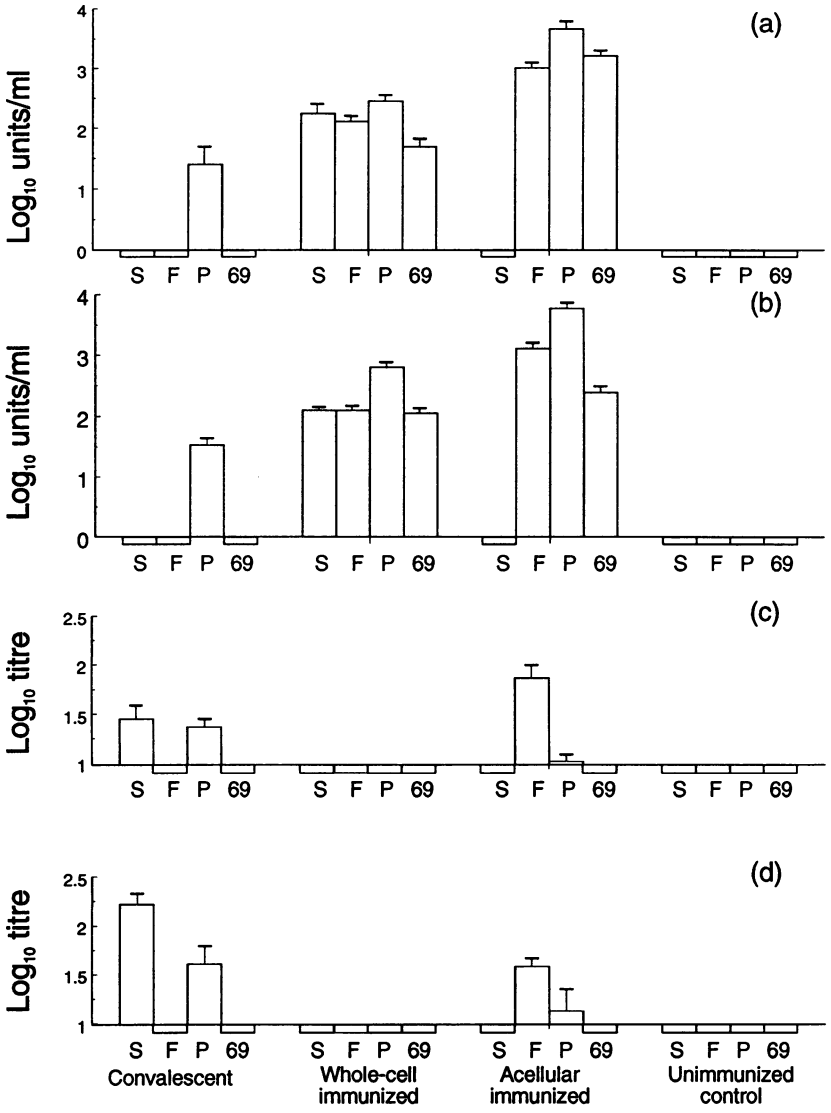

FIG. 3. Antibody responses to *B. pertussis* components after an aerosol challenge. Shown are responses of convalescent, WCV-immunized, acellular vaccine-immunized, and nonimmunized mice to *B. pertussis* sonicate (S), FHA (F), PT (P), or pertactin (69). Serum IgG antibody levels were measured 5 days (a) and 15 days (b) after the challenge. Lung IgA antibody levels were measured 5 days (c) and 15 days (d) after the challenge. A minimum of six mice from each group at each time point were assayed. Bars represent standard errors of the means.

responses following a respiratory infection are disparate. In naive mice, a reasonable T-cell response is detectable within 2 weeks of infection, and 3 weeks later, when the bacteria have been cleared, this response is very strong, whereas the humoral response is only just detectable. Furthermore, we have also demonstrated that adoptive transfer of immune $CD4^+$ T cells from convalescent mice can confer protection against a subsequent challenge in the absence of a detectable antibody response (24). Therefore, the induction of CMI is obviously crucial in the elimination of an infection in naive mice and in mice rendered immune following a respiratory infection. However, the role of antigen-specific T cells in acquired immunity generated following immunization has not been investigated.

The results of the spleen cell lymphokine secretion assays provided indirect evidence for the induction of different T-cell subpopulations by a *B. pertussis* infection or immunization. Recent findings have pointed to the existence of subsets of murine helper T cells, named Th1 and Th2, distinguishable by the array of lymphokines they secrete (7, 26). The secretion of high levels of IL-2 and INF-γ and low levels of IL-4 and IL-5 by spleen cells from convalescent mice was typical of a Th1 response, whereas acellular vaccine-immunized mice produced a lymphokine secretion profile more typical of a Th2 response, with high IL-5 and low IL-2 and IFN-γ levels (27). Although spleen cells from immunized or infected mice all produced low levels of IL-4, *B. pertussis*-specific T-cell lines derived from mice immunized with the acellular vaccine produced high levels of IL-4 and IL-5 (2). This observation is consistent with the demonstration that in vitro restimulation is often required to detect IL-4 production (51) and confirms the fact that the acellular vaccine induces predominantly Th2 cell types. Th1 cells are predominantly effective in mediating inflammatory, cytotoxic, and delayed-type hypersensitivity responses, and Th2 cells are more efficient in providing help for antibody pro-

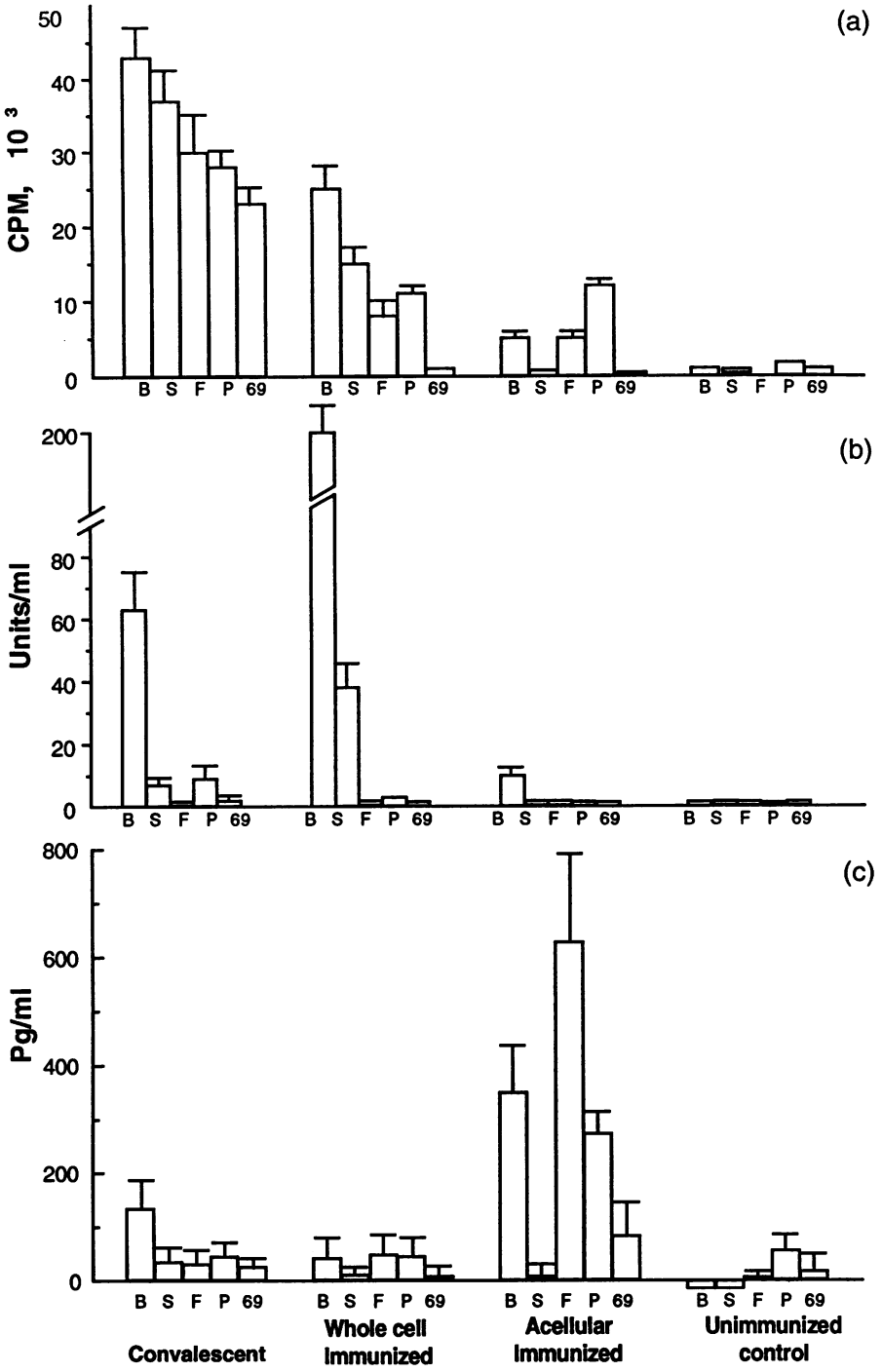

FIG. 4. Lymphokine release from spleen cells after in vitro stimulation with *B. pertussis* components. IL-2 (a), IFN-γ (b), and IL-5 (c) levels were measured in supernatants following stimulation of spleen cells from convalescent, WCV-immunized, acellular vaccine-immunized, and nonimmunized mice with killed *B. pertussis* cells (B), sonicate (S), FHA (F), PT (P), or pertactin (69). A minimum of six mice from each group were assayed. Bars represent standard errors of the means.

duction (3, 7, 26). These facts are consistent with our findings that infection induced strong T-cell proliferation and high levels of IL-2 and IFN-γ production but little antibody production (cellular immunity), whereas immunization with the acellular vaccine led to a strong antibody response and the production of high levels of Th2-derived cytokines but limited T-cell proliferation (humoral immunity).

Although Th cell subpopulations with distinct cytokine secretion patterns have been more difficult to identify in humans, recent reports have demonstrated the presence of Th1 and Th2 cell types following exposure to infectious agents (8, 40). In a study of T cells from an individual who had suffered from a pertussis infection, Peppoloni et al. (33) reported that T-cell clones specific for PT and other pertussis

antigens secreted IL-2 and IFN-γ but low or undetectable levels of IL-4. Furthermore, these $CD4^+$ T-cell clones displayed cytotoxic function. Therefore, in humans as well as in the mouse model, it appears that protection against a subsequent *B. pertussis* infection may be correlated with a Th1 response. However, our findings also suggest that a strong Th2-enhanced antibody response may be associated with an initially larger reduction in the level of bacterial colonization. The fact that *B. pertussis* is not an exclusively noninvasive pathogen may explain the importance of a Th1 response in immunity to a *B. pertussis* infection. Cellular responses mediated by Th1 cells have been associated with protection against intracellular bacteria (19, 28), and the lymphokines released by Th1 cells have been shown to recruit, stimulate, and activate phagocytic cells, such as macrophages and neutrophils, against infections (21, 47, 50). Such responses may not only eradicate bacteria on the mucosal surface but also destroy infected cells, thus exposing intracellular bacteria to attack by other immune processes. While a Th2 response, through its promotion of a strong antibody response, may block the adhesion of bacteria and neutralize toxin-mediated activities, it would be of limited value in the elimination of bacteria within cells.

The inflammatory and helper activities of $CD4^+$ T cells are now considered to be mutually exclusive and under reciprocal regulatory control (18, 32). It has been shown that IFN-γ produced by Th1 cells inhibits the proliferation of Th2 cells (14), and it has also been shown that IL-10 produced by Th2 cells can inhibit cytokine synthesis, especially IFN-γ production, by Th1 cells (12, 25). It is therefore surprising that mice immunized with the WCV should have a lymphokine secretion profile indicative of a Th1 response and yet produce a relatively high serum antibody response. The antibodies produced by B cells induced with this immunogen may not have required high levels of Th2-derived lymphokines but may have been stimulated by IL-2 or IFN-γ.

There are several possible reasons for the poor efficacy of the acellular vaccine used in this study. Antigen presentation may have been poor because of its nonparticulate form. The chemical detoxification of PT may have eliminated important conformational antibody epitopes or antigen processing sites for T-cell regulation. The use of alum may have favored the induction of a Th2 response, and the subsequent production of IL-4 and IL-10 could have down-regulated the Th1 response, as has been shown for parasite infections (31, 48). Some essential protective antigens may have been omitted, or the major targets of Th1 cells induced by an infection or the WCV may not have been PT, FHA, or pertactin. The strongest cellular immune responses, in particular IFN-γ production, following an infection or WCV immunization were to killed bacteria and a *B. pertussis* sonicate which, in relative terms, probably contained very little PT, FHA, and pertactin. The combination of antigens present in the acellular vaccine used in our study was selected because PT is present in all manufacturers' acellular pertussis vaccines and FHA and pertactin are the next most commonly proposed vaccine components. Although we do not rule out the possibility that PT, FHA, and pertactin may have generated T cells that produced IL-2 and IFN-γ, our results suggest that the present acellular vaccine formulation may not favor the induction of Th1 cells.

Humoral immune responses are probably involved in several aspects of protection against pertussis. The results presented here show a correlation between high levels of serum IgG to *B. pertussis* antigens and the rapid initial clearance of bacteria, which may be due to the transudation of antibodies into the lungs. However, direct cellular immune responses to *B. pertussis* are necessary for complete elimination of the bacteria. It therefore follows that assessment of the possible protective potency of *B. pertussis* components should be based on more than just serological responses. Clinical trials of acellular pertussis vaccines should be designed to include the study of cellular as well as humoral immune responses to expand the search for immune correlates of protection. The development of more effective acellular pertussis vaccines should encompass the induction of cellular and humoral responses.

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